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# Single Spin-Echo $T_2$ Relaxation Times of Cerebral Metabolites at 14.1 T in the *in vivo* Rat Brain

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Word count: 3349
Number of figures: 3
Number of tables: 1
Number of references: 20

**Abstract**

Object

To determine the single spin-echo $T_2$ relaxation times of uncoupled and J-coupled metabolites in rat brain *in vivo* at 14.1 T and to compare these results with those previously obtained at 9.4 T.

Materials and Methods

Measurements were performed on five rats at 14.1 T using the SPECIAL sequence and TE-specific basis-sets for LCModel analysis.

Results and Conclusion

The $T_2$ of singlets ranged from 98 ms to 148 ms and $T_2$ of J-coupled metabolites ranged from 72 ms (glutamate) to 97 ms (myo-inositol). When comparing the $T_2$s of the metabolites measured at 14.1 T with those previously measured at 9.4 T, a decreasing trend was found ($p < 0.0001$). We conclude that the modest shortening of $T_2$ at 14.1 T has a negligible impact on the sensitivity of the $^1$H MRS when performed at TE shorter than 10 ms.

**Keywords**: $T_2$ relaxation time, coupled spin systems, brain metabolites, proton magnetic resonance localized spectroscopy.

## Introduction

In vivo $^{1}$H magnetic resonance spectroscopy (MRS) allows for non-invasive investigations of neurochemical information and thus has been widely used for studies of cerebral diseases such as Alzheimer's disease, schizophrenia, stroke and others [1-3]. To preserve the maximum signal, a short TE is preferable for minimizing signal loss due to $T_2$ relaxation and signal modulation due to J-coupling. As an alternative, a moderate/long TE is employed to eliminate the confounding macromolecule signal from the $^{1}$H MR spectra or to optimize the detection of specific metabolites of interest [4]. At a moderate/long TE, quantification of J-coupled cerebral metabolites is possible when taking into account the spectral shape modulation due to J-coupling, and the signal loss due to $T_2$ relaxation time.

The evaluation of the $T_2$ relaxation time has been mostly performed for singlet resonances such as that of the N-acetylaspartate acetyl moiety (at 2.01 ppm), total creatine (at 3.03 ppm and 3.93 ppm) and choline-containing compounds (at 3.2 ppm) [5,6]. For J-coupled metabolite resonances, the modulation with TE due to J-coupling represents a confounding factor in the assessment of the signal loss due to $T_2$. In a previous study [7], the feasibility of measuring $T_2$ of J-coupled metabolites in rat brain at 9.4 T was demonstrated by using the LCModel [8] with TE-specific basis sets, which take into account the signal modulation due to J-coupling with TE. The same methodology was further used for measuring $T_2$ of J-coupled metabolites in human brain at 3T [9], 4T [10] and 7T [11].

To achieve a higher sensitivity and spectral resolution in $^{1}$H MRS, the field strength ($B_0$) of MR scanners is steadily increasing, with an increasing number of scanners for preclinical research being installed at $B_0$ of 9.4 T and higher. A significant reduction in

the $T_2$ values of proton resonances (water, N-acetylaspartate, and total creatine) has been observed with increasing magnetic field strength and has been attributed mainly to increased dynamic dephasing due to increased local susceptibility gradients [12]. Since the quantitative contribution of different relaxation mechanisms is not clearly understood in the *in vivo* tissue, the relaxation times need to be determined experimentally at these new field strengths. Therefore, the current study carries a two-fold purpose: first, to determine the single spin-echo $T_2$ of uncoupled and J-coupled cerebral metabolites in rat brain *in vivo* at 14.1 T; second, to compare these results with those obtained in the previous study performed at 9.4 T [7].

## Materials and Methods

### *Animal and phantom preparation*

In vivo MRS experiments were performed on five male healthy Sprague Dawley rats (250-300 g), which were anesthetized by 1.5 – 2 % isoflurane in oxygen during the experiment. The animal was placed in a homebuilt holder and the head was stereotaxically fixed by a bite bar and a pair of ear bars. Respiration rate was monitored by a small animal monitor (SA Instruments Inc., NY, USA) and maintained at 70 - 100 breaths/min. The body temperature was measured by a rectal thermosensor and maintained at 37.5 ± 0.5 $^{o}$C with the circulation of heated water. No blood gas measurement was performed during the experiment. All animal preparation procedures were performed according to local and federal guidelines and the protocols were approved by the local ethics committee.

### *In vivo MR experiments*

All MRS experiments were carried out on a DirectDrive MR spectrometer (Varian, Palo Alto, CA, USA) interfaced to a 14.1 T, 26 cm horizontal bore magnet (Magnex Scientific, Oxford, UK). A home-built $^{1}$H quadrature surface coil consisting of two geometrically decoupled 14 - mm - diameter single loops was used as both a transmitter and receiver.

In vivo $^{1}$H MR spectra were acquired from a VOI (5 × 2.5 × 5 mm$^{3}$) centered in the hippocampus, using a spin-echo based full intensity localized pulse sequence (SPECIAL [13]) with the following parameter settings: TE = 2.8, 20, 40, 60, 80, 110, 130, 150, 170, 200, and 300 ms, TR = 4 s, spectral bandwith = 7 kHz, 4096 complex points and 10 FID data block each TE (16 averages/block). Spectra were acquired with increasing TEs and total experiment lasted about 2 hours. The VAPOR water

suppression technique [14] was applied in an interleaved way with series of outer volume suppression pulses. The static field inhomogeneity was optimized by first- and second-order shims using echo-planar imaging-based FASTMAP [15], which resulted in a water signal linewidth of 16 - 19 Hz. For fitting peak intensities, high-SNR spectra were used (the mean SNR of the NAA methyl peak was ~ 7 at the longest TE of 300 ms). In vitro $^{1}$H MR spectra were acquired with a similar protocol on a phantom containing creatine (50 mM) and glutamate (50 mM).

***Data Analysis***

To determine the single spin-echo $T_2$ of J-coupled metabolites, the J-modulation of the lineshapes was taken into account by using LCModel with TE-specific basis sets that were used for analyzing the spectra acquired at each TE [7]. Nineteen metabolites (alanine, aspartate, γ-aminobutyric acid, glutamine, glutamate, glutathione, glycine, myo-inositol, lactate, scyllo-inositol, taurine, ascorbate, glucose, N-acetylaspartylglutamate, phosphorylethanolamine, glycerophosphorylcholine, N-acetylaspartate, creatine and phosphocreatine) were included in the basis sets and the corresponding spectra were generated with density matrix simulations [16], using published values of coupling constants J and chemical shifts δ [17] ($T_2$ relaxation was not included). Subspectra of the acetyl and aspartate moieties of N-acetylaspartate (NAA), and the $CH_3$ and $CH_2$ groups of total creatine (tCr), were prepared as separate components in the basis sets for measuring $T_2$ of individual chemical groups in the same molecule. Macromolecule spectra were obtained as previously described [18] and included as one component in the basis sets of each TE. Metabolite concentrations obtained after the LCModel quantification were fitted to a monoexponential function

using curve-fitting tool in Matlab (Version 7.1, The MathWorks, Inc., Natick, MA, USA). The quality of the $T_2$ fit was assessed by the coefficient of determination ($R^2$). Only the $T_2$ of metabolite resonances with $R^2 > 0.95$ are reported here.

**Results**

In spectra measured *in vitro*, the singlet resonances of creatine at 3.03 ppm and 3.92 ppm decayed in amplitude with increasing TE, while resonances of J-coupled metabolites i.e. glutamate (Glu) displayed both a decrease in amplitude and a modulation in their shape, e.g., the resonances at 2.34 ppm refocused at TE = 110 ms (data not shown). Spectral modulations of glutamate were successfully mimicked by spin simulations (data not shown), which validates the accuracy of the spectral simulations.

*In vivo* water-suppressed $^{1}$H MR spectra and their corresponding LCModel fits show excellent correspondence at different TEs (Fig. 1). The glutamate $CH_2$ multiplet at 2.34 ppm and the myo-inositol (Ins) resonance peaks at 3.61 ppm developed from an absorption shape (at TE = 2.8 ms) to a dispersion shape (at TE = 40 ms) and then again to an absorption line appearance (at TE = 80/110 ms) (Fig.1). The high SNR at 14.1 T allowed for reliable measurement of tCr, total choline (tCho) and NAA singlet resonance with CRLB < 5%, and Ins, taurine (Tau), Glu and glutathione (GSH) with CRLB < 30% at most TEs.

Curve fitting of apparent concentrations for singlets (NAA $CH_3$ at 2.01 ppm) and J-coupled metabolites (Ins, Glu) suggested that the decrease of signal intensities with TE is largely described by a mono-exponential decay (Fig. 2). The $T_2$ of singlets ranged from 98 ms to 148 ms, where the $T_2$ of tCr ($CH_2$) is the shortest for singlets and the $T_2$ of tCho (dominated by methyl group) is the longest. For J-coupled cerebral metabolites, $T_2$ ranged from 72 ms (Glu) to 97 ms (Ins, Table 1).

When comparing the $T_2$s of the metabolites measured at 14.1 T with those previously measured at 9.4 T [7], a decreasing trend was found ($p < 0.0001$, two-way ANOVA)(Fig. 3). However, the decrease in $T_2$ was rather small (on average 10%) except for NAA (30%) and Ins (38%) (Fig. 3).

## Discussion

The present study reports the $T_2$ relaxation times of singlets and J-coupled metabolites including NAA($CH_3$), tCr($CH_3$), tCr($CH_2$), tCho, Ins, Glu, GSH and Tau at 14.1 T using single spin-echo excitation and LCModel analysis with TE-specific basis-sets.

Both in vitro and in vivo spectra (Fig. 1) demonstrated that metabolites with singlet resonances decrease with TE in spectral amplitude while J-coupled metabolites such as Glu, vary with TE in both amplitude and spectral pattern. When considering the spectral pattern modulation with TE in the basis sets for LCModel analysis, the apparent concentration decays mono-exponentially with TE due to signal loss of $T_2$ relaxation time (Fig. 2), which allows for the quantification of the $T_2$ relaxation times of J-coupled metabolites.

Since the signal intensity (i.e., the integral of resonance peaks) varies with TE for J-coupled metabolites, spectra measured at all TEs cannot be optimal for the $T_2$ quantification of all metabolites. For instance, the signal intensity of Glu and Ins peaks were close to zero around TE = 40, 60 ms and TE = 40, 130, 150 ms, respectively, which might decrease the measurement accuracy. Therefore, apparent concentrations obtained at those TEs in fit plots (Fig. 2) demonstrate larger deviations from the fitting curve. However, when excluding those spectra from the fit data, the precision of the fit was improved, while no substantial difference was found for $T_2$ values (within the fitting error).

Data acquisition was performed with SPECIAL, which is a localization method based on a single spin-echo acquisition scheme. During the inter-pulse delay, magnetization components could not be completely refocused by the refocusing pulse due to dynamic dephasing induced by diffusion in the inhomogeneous local magnetic field and chemical exchange [12]. Therefore, signal loss increased with the length of TE and thus reported $T_2$ values in this study were shorter than the intrinsic $T_2$ relaxation times. $T_2$ values of well-represented metabolites such as NAA($CH_3$), tCr and tCho measured at 14.1 T in this study were shorter than those reported at 4.7 [19], 9.4 and 11.7 T [6] in healthy rat brain. The reduction of $T_2$ relaxation times with increasing magnetic field strength can be ascribed to the increased loss of spin coherence due to molecular diffusion across local magnetic field gradients, which are proportional to the magnetic field strength. The contribution of chemical exchange might be negligible for non-exchanging protons of NAA($CH_3$), tCr and tCho.

The measured apparent $T_2$ relaxation times vary with the pulse sequence applied. The CPMG-type of sequence such as LASER or CP-LASER suppresses signal dephasing and results in a longer $T_2$ values of metabolites compared to PRESS or single SE sequence [12]. In addition, the local magnetic gradient could be altered by the level of blood oxygenation due to different tissue composition and physiology, which leads to the change in $T_2$. For avoiding the aforementioned concern in comparing $T_2$ values at 14.1 T to those previously reported at 9.4 T, the measurement at 14.1 T was performed on an identical Varian INOVA spectrometer using the same acquisition protocols (the pulse sequence with the same RF pulses and TEs), RF surface coils with the same shape and size, the VOI having similar tissues composition, and the same data analysis

(LCModel fit with TE-specific basis-sets) as in the previous measurement at 9.4 T. Although the blood gas was not monitored during the experiment, the respiration rate was maintained within the physiological range, the same way in both 9.4 T and 14.1 T experiments. Therefore, $T_2$ difference introduced by different degree of blood oxygenation should be negligible and any deviations among individual animals should be averaged out.

The modest decrease in $T_2$s at 14.1 T compared to those we previously reported at 9.4 T (Fig. 3) is consistent with previous studies [12,6]. The degree of $T_2$ changes might reflect different microenvironment or chemical exchange contribution of individual metabolites, which leads to different degree of dynamic dephasing.

The decrease in $T_2$ with increasing field strength can impact the SNR increase that is gained when performing MRS experiments at ultra high magnetic field. Note that despite the shortened $T_2$ at 14.1 T, the mean $T_2$ of metabolites (104 ms) at 14.1 T leads to a signal loss of 10%, when using TE = 10 ms, which is within the biological variation. Therefore, the effect on the metabolite quantification errors and SNR is expected to be negligible when using a TE < 10 ms.

For the quantitation of metabolites at a long TE, such as, for example, in editing techniques [20] an internal reference (tCr methyl group) was sometimes employed when assuming similar $T_2$ impact on edited metabolites and the internal reference. At 14.1 T, the difference in $T_2$s of J-coupled metabolites (Glu, Tau, Ins and GSH) and that of the tCr methyl resonance should be taken into account when tCr methyl peak is used as a

reference in the quantification of J-coupled metabolites using long-TE editing technique.

## Conclusion

We conclude that the $T_2$ relaxation times of uncoupled and coupled cerebral metabolites in rat brain at 14.1 T showed a decreasing trend compared to 9.4 T, however, the impact is negligible on the quantification of neurochemical profile performed at short echo times.

## Abbreviations used:

tCho, total choline (Glycerophosphorylcholine + Phosphorylcholine); tCr, total creatine (phosphocreatine + creatine); Cr, creatine; GSH, glutathione; Glu, glutamate; Ins, myo-inositol; NAA, N-acetylaspartate; Tau, taurine; VOI, volume of interest; SPECIAL, SPin ECho, full Intensity Acquired Localized spectroscopy.

## Acknowledgements

This study was supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL, the Leenaards and Jeantet Foundations.

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Table 1. $T_2$ relaxation times (mean ± sd) of metabolites in rat brain at 14.1 T. $R^2$ is the coefficient of determination (tCho = total choline, NAA = N-acetylaspartate, tCr = total creatine, Ins = myo-inositol, GSH = glutathione , Tau = taurine, Glu = glutamate ).

| **Metabolite** | **$T_2$ (ms)** | **$R^2$** |
|---|---|---|
| NAA($CH_3$) | 142 ± 7 | 0.988 |
| tCr($CH_3$) | 107 ± 2 | 0.998 |
| tCr($CH_2$) | 98 ± 2 | 0.996 |
| tCho | 148 ± 14 | 0.958 |
| Ins | 97 ± 6 | 0.983 |
| GSH | 87 ± 13 | 0.948 |
| Tau | 82 ± 7 | 0.965 |
| Glu | 72 ± 8 | 0.958 |

## Figure caption

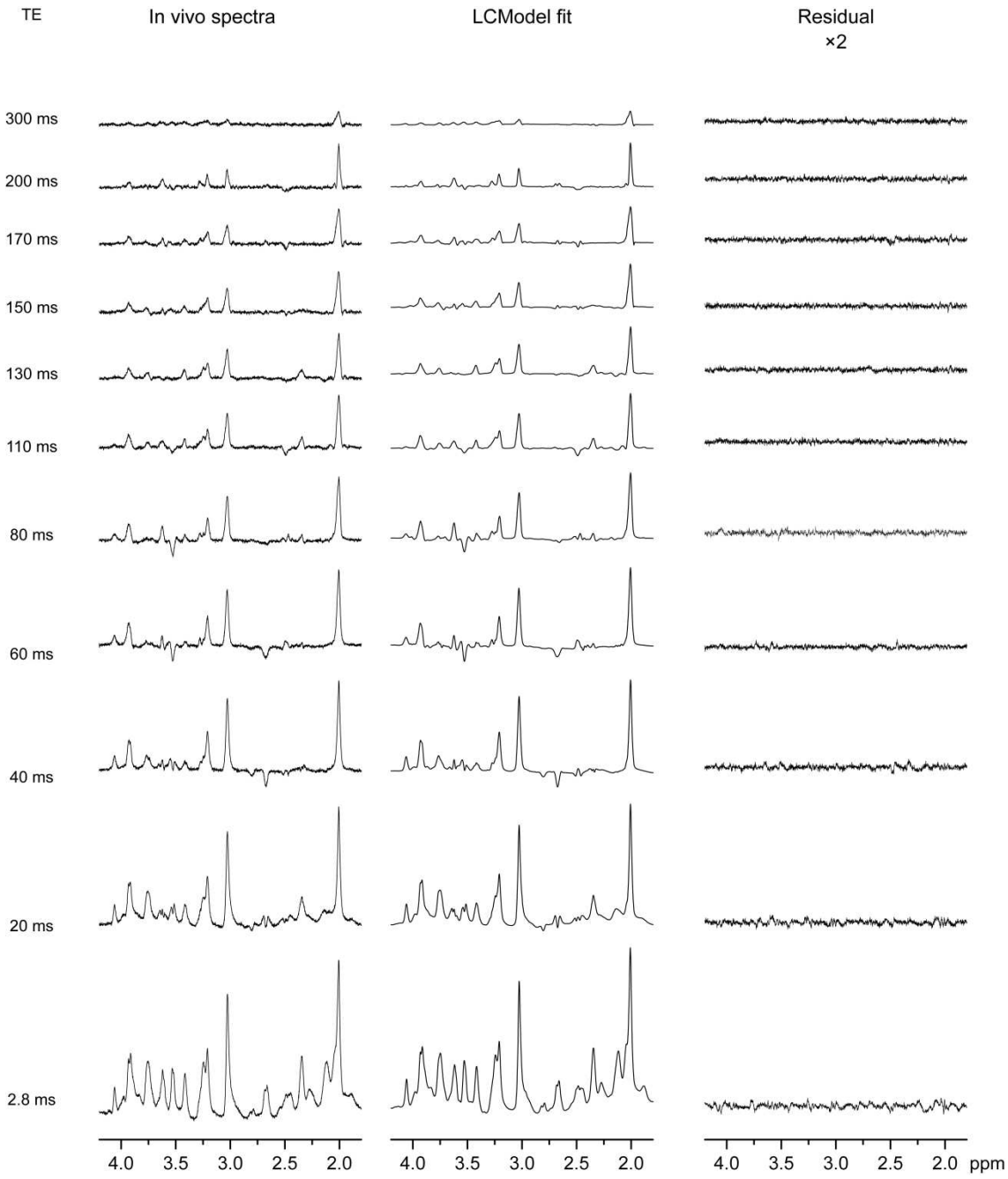


Fig. 1. A series of in vivo spectra, the corresponding LCModel fits and the fitting residuals for TE ranging from 2.8 ms to 300 ms.

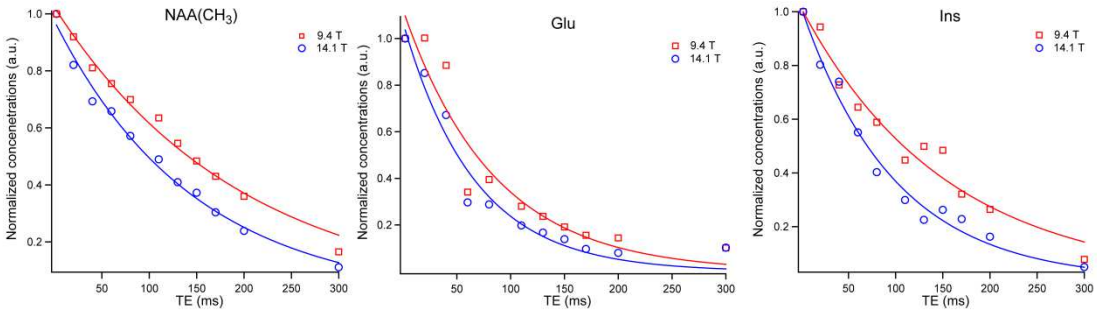


Fig. 2. Plots of the normalized apparent concentration as a function of TE of the NAA singlet, myo-inositol and glutamate at 14.1 T (circles) and 9.4 T (squares). Solid lines represent the exponential fit. Data points for 9.4T were taken from previously published study [7].

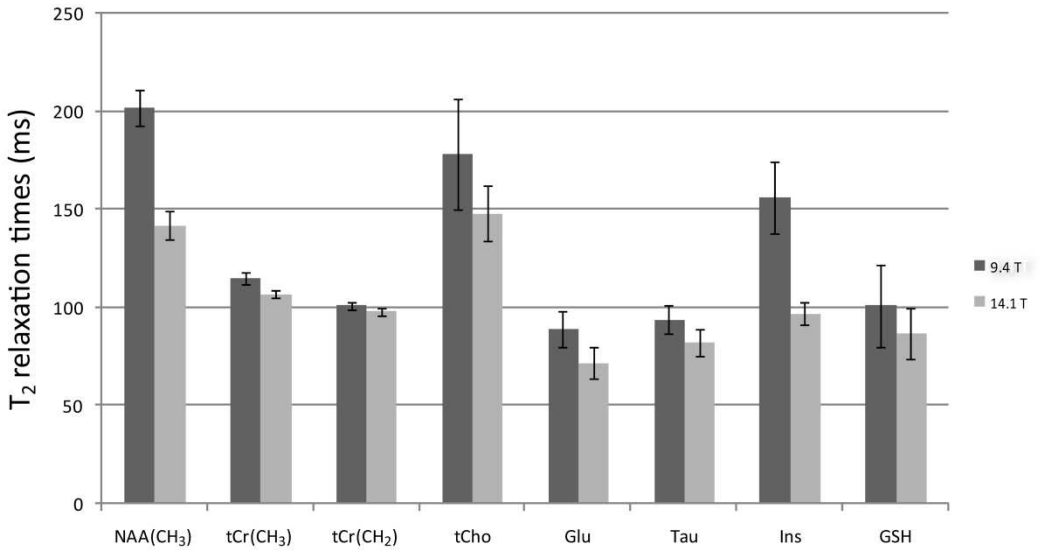


Fig. 3. $T_2$ relaxation times (mean ± sd) of metabolites resonances measured at 14.1 T and 9.4 T (from previous study [7]) using same data acquisition parameters and data analysis methods (LCModel fit with TE-specific basis-sets).